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On the action of beryllium on alcaline phosphatase.

by Jean Roche, Ngyuyen Van Thoai and Jacqueline Loewy

It has been recently noted that beryllium slts show, with respect to alcaline phosphatase (phosphomonoesterase), an energetic inhibiting action, to which some have tried to connect the mechanism of certain industrial poisons (1*, 2*, 3*). The inhibition caused by Be^{++} would be due to a competition with magnesium, a natural component of the enzyme; its study from this point of view, presents a particular interst concerning the action machanism of metals on phosphatase. Indeed, many divalent cations, among which are Mg^{++}, Mn^{++}, and Zn^{++}, reactivate it (the p osp atase) after its inhibition by prolonged dialysis and are in this regard interchangeable (3*,4*). Each of these ions, then, show a maximal activity at a particular concentration, very weak in the case of some (1.10^{-5} with zinc salts), above which they are even better inhibitors according to the height of their rate in the medium. The different cations enter into competition to reactivate the dialysized enzyme accumulated from their mixture and their respective relationships to phosphatase, therefore determining the degree of activation of the phosphatase. Similarly, when the enzyme has been activated by a single cation of optimal concentration, the addition would, by itself, reactivate the dialysized phosphatase. It was important to study to what extent the action of beryllium is different from that of other divalent metals, and if, as has been admitted, the first is uniquely an inhibitor with respect to alcaline phosphatase; the mechanism of its action would the be different to that which belongs to an assembly of cations.

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Our research has a bearing on the preparation of intestinal phophatase (dog) of a heightened degree of purification (5*) and on the preparation of raw renal enz yme (pig), (Alber's Technique, see 6*). The enzymes were dialized at pH= 4.6 and at 37° to the point of nearly total inactivation, then alanine 1 10^{-2}M at pH=8.8 was added and they were placed for 2 hours at 37° in the presence or in the absence of magnesium sulfate 1.10^{-1}M, with or without the addition of beryllium sufate (SO_4Be, $4H_2O$), at the concentrations between 1.10^{-5}M to 1.10^{-3}M inclusive. Their activity at pH= 8.8 on the -glycerophosphate of sodium M/30 was determined in the conditions adopted on our previous research (5*) and we calculated on the bases of the experimental results of the percentages of activition or inhibition with respect to evidence wherein the enzyme was incubated in the presence of pure alanine. Comparative experiments were begun which substitued zinc acetate for beryllium sulfate in order to show the analogy or the diversity of the action of the ions Be^{++} and Zn^{++}, the activation of the latter manifested itself only at very minimal rates. You will see in the Tables below a few examples of the values obtained.

The ion beryllium is therefor, along with the zinc ion and several other divalent cation, likely to reactivate the alcaline phosphatase (dialysized) when it is placed in the presence of the phosphatase at very weak concentrations (1.10^{-5}M). At progressively higher rates, it provokes a lesser activation, then an inhibition of the enzyme. In the presence of magnesium salts, the addition of beryllium ions leads. as does the addition of zinc ions, to an inhibition which can be linked to a phenomenon of competition between the two metals (5). The action of beryllium salts is not therefore specifically inhibiting; its value

and intensity are factors of the conditions under which it is working.

Conclusions: The beryllium ion, energetic inhibitor of alcaline phosphatase when the metallic component of the phosphatase has not been disassociated, reactivates the enzyme after prolonged dialysis. It follows from this that, id the Be^{++} ion is an energetic inhibitor of alcaline phosphatase in the tissues and their extracts, the modalities of its action on the purified enzyme are identical to those of other cations, particularly Zn^{++}. The interchangeability of divalent metals in the reactivation of alcaline phosphatase and their competition in the reconstitution of the enzyme molecule disassociated through dialysis appear therefore to be general.

(Genreal and comparative biochemistry, College of France.)

Footnotes:

(1*) F.W. Klemperer, J. M. Miller and C.J. Hill, J.biol. Chem., 1949, t. 180, p.281

(2*) R.S. Grier, M.B. Hood and M.B. Hoagland, J. biol. Chem., 1949, t. 180, p.289

(3*) A. Policard and A. Fulleringer, C.R. de la Soc de Biol., 1949, t. 143, p. 1411.

(4*) J. Roche, Nguyen van Thoai and J. Durand, Bull. Soc. Chim. biol., 1943, t. 25, pp. 1166 and 1175.

(5*) Nguyen van Thoai, J. Roche and M. Roger, Biochim. Biophys. Act., 1947, t. 1, p.61.

(6*) D. Albers, Ztschr. f. Physiol. Chem, 1940, T. 265, p. 129.

TABLE I.

(a) Origine de la phosphatase et n° de la préparation	(b) Effecteur et concentration moléculaire de celui-ci	(c) Pourcentage d'activation ou d'inhibition
(d) Intestin I.....	SO_4Be 1.10^{-3} M	+ 88
	1.10^{-4} M	+ 22
	1.10^{-5} M	+ 22
	SO_4Zn 1.10^{-3} M	+ 78
	1.10^{-4} M	+ 53
	1.10^{-5} M	− 35
Intestin II.....	SO_4Be 1.10^{-3} M	+ 20
	1.10^{-4} M	+ 6
	1.10^{-5} M	− 29
Intestin III.....	SO_4Be 1.10^{-3} M	+ 45
	1.10^{-4} M	− 17
	1.10^{-5} M	− 43
(e) Rein I	SO_4Be 1.10^{-3} M	+ 30
	1.10^{-4} M	+ 6
	1.10^{-5} M	− 20

a. Origin of phosphatase and no. of preparation.

b. Actvator and its molecular concentration.

c. Percentage of activation or inhibition.

d. Intestine. e. Spine.

Caption: Percentage of activation (+) or inhibition (-) by beryllium sulfate and zinc acetate on alcaline phosphatase (purified as in intestine), or raw (as in spine) and inactivated by dialysis at pH= 4.6 (incubation at pH= 8.8 and at 37° for 2 hours in the presence of alanine 1.10^{-2}M added from the activator).

TABLE II.

a. Origine de la phosphatase et n° de la préparation	b. Effecteur et concentration moléculaire de ceux-ci	c. Pourcentage d'activation
d. Intestin I.....	SO^4Mg 1.10^{-1} M	+378
	Id. + SO^4Be 1.10^{-3} M	+238
	» + SO^4Be 1.10^{-4} M	+205
	» + SO^4Be 1.10^{-5} M	+133
	» + SO^4Zn 1.10^{-3} M	+336
	» + SO^4Zn 1.10^{-4} M	+309
	» + SO^4Zn 1.10^{-5} M	+238
e. Rein I	SO^4Mg 1.10^{-1} M	+166
	Id. + SO^4Be 1.10^{-3} M	+141
	» + SO^4Be 1.10^{-4} M	+125
	» + SO^4Be 1.10^{-5} M	+105

a. Origin of phosphatase and no. of preparation

b. Activator and its molecular concentration

c. Percentage of activation or inhibition

d. Intestine.

e. Spine

Caption: Percentage of activation (+) or inhibition(-) by beryllium sulfate and zinc acetate or alcaline phosphatase (purified as in intestine) or raw (as in spine) and inactivated by dialysis at pH= 4,6 (incubation at pH= 8.3 and at 37° for 2 hours in the presence of alanine 1.10^{-2}M added from the activator).